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# Stimulation of Indigenous Carbonate Precipitating Bacteria for Ground Improvement

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**Abstract.** Calcite minerals are precipitated in soil through biomineralisation which can be either organic or inorganic in nature. Biomineralisation can be employed to improve ground conditions in its natural state. Usually, studies of applied biomineralisation are highly interdisciplinary involving expertise from engineers, chemists and microbiologists. In this paper, we study the potential of biomineralisation from indigenous bacteria present in soil. The soil samples were collected from a high permeable zone and the bacteria that inhabit the soil were stimulated at a temperature of 15 °C. A cementation solution consisting of 500mM calcium chloride, urea and nutrient broth at a pH of 7.5 was added to the soil samples. Inorganic precipitation was found to be dominant and was more efficient when compared to organic precipitation. Carbonate precipitation data indicated that inorganic precipitation were 1.37 times better at carbonate formation in comparison to organic precipitation. Scanning Electron Microscopy analysis identified cementation bonds formed between soil particles. It was deduced that organic precipitation is dependent on temperature, and may take an extended time at such low temperature. The preliminary data presented in this paper suggests that the implementation of biomineralisation with in-situ microbes is promising but requires further laboratory and field investigation before being considered for engineering application.

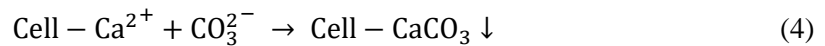
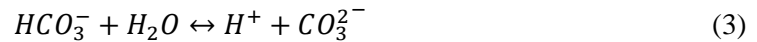
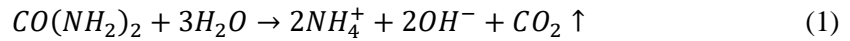
## 1. Introduction

Bacterial application towards engineering purposes is attracting the attention of microbiologist and geotechnical engineers worldwide. Biomineralisation has recently gained popularity among researchers for its applicability towards ground improvement applications. Microbial activity that alters the chemical environment favoring mineral formation is known as Biomineralization [1], [2]. Microbially induced calcite precipitation (MICP) is a biochemical mechanism which is driven by the microorganism upon interacting with a chemical solution rich in calcium. Research in MICP has shown that microbially released CO<sub>2</sub> interacts with the biomineralization solution favoring carbonate formation. The carbonate combines with the calcium ion (Ca<sup>2+</sup>) leading to the precipitation of calcium carbonate.

In the biomineralization process, bacteria commonly serve as nucleation sites for the precipitation of calcium carbonate. One of the important enzymes that's been shown to mediate high percentage of carbonates is urease. Urease (UA; EC 3.5.1.5) is a nickel-containing metalloenzyme found in a wide range of microorganisms [3]. Presence of urease initiates a process commonly known as ureolytic activity which aides in the conversion of urea to ammonia and CO<sub>2</sub> (Equation 1). Ureolytic activity promotes superior precipitation of carbonate (Equation 2 and 3). Ca<sup>2+</sup> ions bind to the negatively charged bacteria surfaces, thus creating a neutral environment for Ca<sup>2+</sup> adsorption (Equation 4).



Bacterial cells are very important for the precipitation of  $\text{CaCO}_3$ , because the bacteria provide nucleation sites (heterogeneous nucleation) and affect the specific types of minerals formed (Figure 2). Okwadha and Li [4] found that high concentration of bacterial cells increases the amount of calcite precipitation by MICP, which happens because of the increase in the urease concentration for urea hydrolysis.



Inorganic precipitation involves the precipitation of minerals in the absence of bacteria and relies heavily on the available atmospheric  $\text{CO}_2$  (Equation 2).  $\text{CO}_2$  helps in the formation of bicarbonate (Equation 6), which later forms calcium carbonate (Equation 3).

Studies on abiotic systems identified calcite precipitation to be less effective in comparison to bacterial system [1]. The precipitation rate is especially high during the early stages of the precipitation process. Since bacteria take time to adjust to the environmental conditions, an abiotic approach could possibly be superior where pretreatment has not taken place.

This paper presents a study that investigates the carbonate precipitating bacterial stimulation from a soil sampled from Formby, UK and the main objective is to provide a preliminary analysis on the possibility of *insitu* application of biomineralisation towards ground improvement.

## 2. Materials and methods

### 2.1 Sample collection

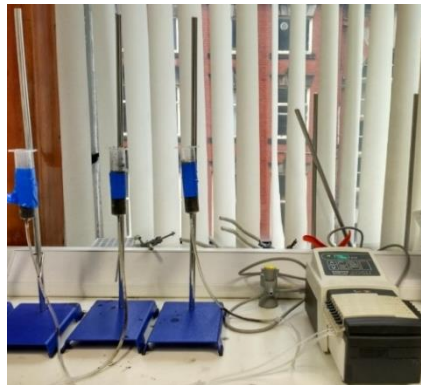
The soil sample was collected from Formby national park, United Kingdom. The soil was collected at 30cm depth using an auger and transported to the lab inside zip lock bags. The soil was stored at 4 °C prior to analysis.

### 2.2 Experimental system

Soil was added to a 50ml syringe column and liquid exchange was performed using a peristaltic pump with a 10 mm ID tube at 5 rpm (Figure 1). Cementation liquid consists of 500mM of Calcium chloride and Urea. Nutrient broth was added along with the calcium chloride and urea. The liquid exchange was performed every 2 days. The experiment was conducted at 15 °C. The experiment was terminated when no liquid was collected during the liquid exchange. The experiments were performed in triplicates to study organic and inorganic precipitation. For inorganic precipitation, the soil was autoclaved to remove biological matter.

### 2.3 Carbonate titration and SEM imaging

Carbonate titration was performed using the protocol followed by [5] and SEM imaging was performed using the Zeiss EVO50 scanning electron microscope at the University of Wolverhampton.

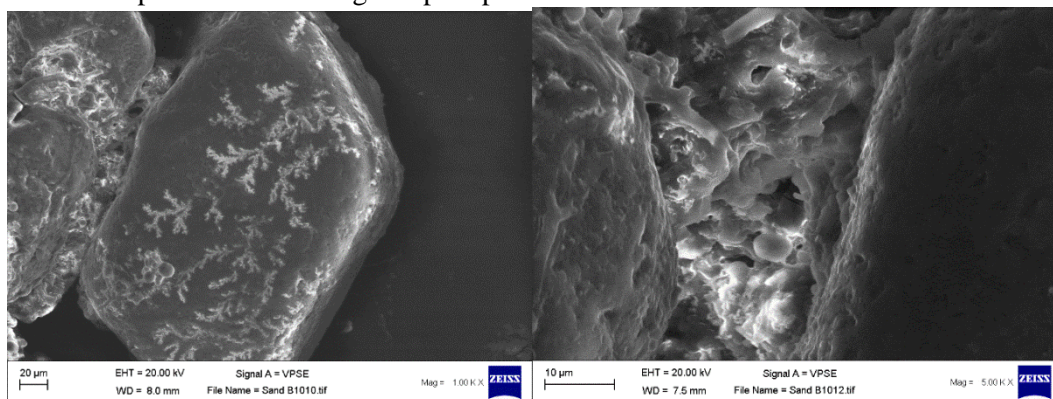


**Figure 1.** Experimental setup for the precipitation process.

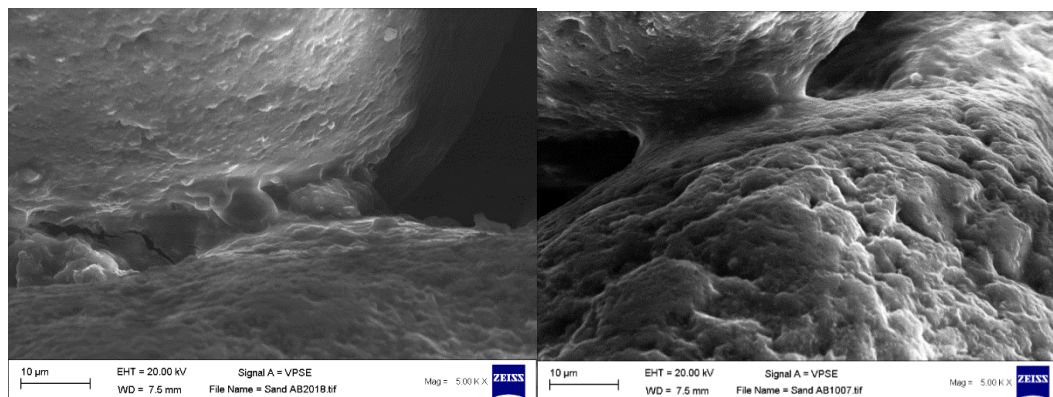
### 3. Results and discussion

The experiments for organic and inorganic precipitation was conducted at 15 °C. A temperature of 20 °C is normally required to obtain an efficient calcite precipitation [6], [7]. However, the experiments conducted for this study were designed to mimic real *in situ* conditions, thus the lower temperature of 15 °C was maintained.

SEM images of particles after mineralization were used to identify the differing precipitation patters between the organic and inorganic samples (Figure 2 and 3). The organic precipitation produced snowflake like crystal formations on the top of the soil particles, such patterns being absent in the inorganic samples. The cementation bonds observed between soil particles due to organic precipitation are denser in comparison to the inorganic precipitation.

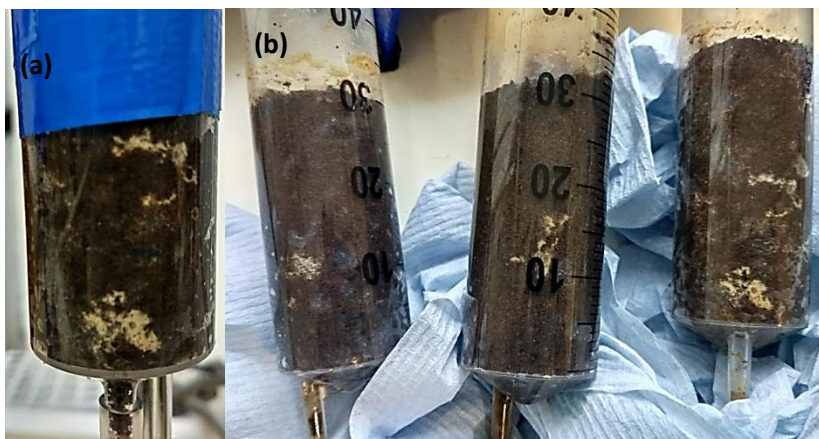


**Figure 2.** SEM images showing the cementation bonds through organic precipitation process.

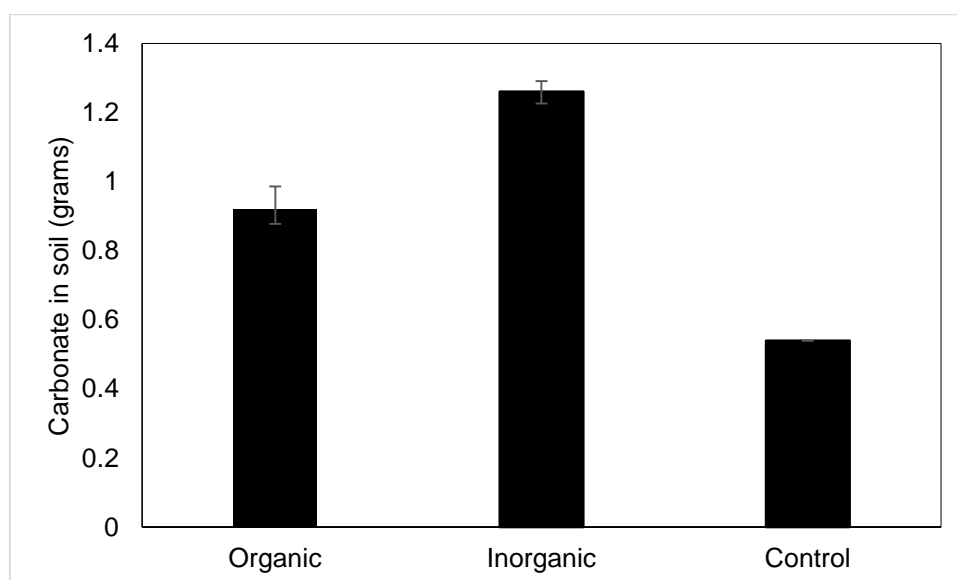


**Figure 3.** SEM images showing the cementation bonds through inorganic precipitation process.





**Figure 4.** Calcium carbonate formations observed between soil particles during organic precipitation experiment (a) and observed in all three columns after the termination of experiments (b). Before the drying of soil samples for carbonate titration, visible calcium carbonate formations were visible in the organic precipitation experiments. This was not observed for the inorganic precipitation experiments.



**Figure 5.** Carbonate precipitation obtained from organic, inorganic and control soil experiments. All experiments were performed in triplicates.

Carbonate titration identified that inorganic precipitation process precipitated 1.37 times more carbonate than the organic precipitation (Figure 5). The data comparison between the organic precipitation and control sample reveals that carbonate precipitating bacteria exist in the soil. The superior precipitation of carbonate through inorganic process is probably due to the additional time required for the organic process, since the bacteria take time to adjust to the environment.

#### **4. Improvements that need to be made for future soil experiments**

The preliminary data presented in this paper suggests that carbonate precipitating bacteria exists in soils and they could be stimulated for calcite precipitation. However, in this case, the organic precipitation was inferior in comparison to the inorganic precipitation, although both were superior to the control. Applied indigenous biomineralisation is still a new technique, a few limitations need to be addressed prior to implementation in the field;

MICP is a microbial processes which highly depends on temperature, pH, calcium concentration, DIC and the presence of nucleation sites [8]. This makes it a complex and time consuming process compared to the chemical process. Bacteria that's present in the soil can produce organic acids to

counterbalance the pH increase associated with ammonia diffusion which results in the delay of crystallization. MICP has to be optimized for time effectiveness before it's used for *insitu* applications. Since the data shown in this study indicates that temperatures as low as 15 °C could make organic precipitation less efficient in comparison to organic precipitation. Multiple parameter studies need to be conducted to optimize the efficiency and crystal productivity for organic precipitation.

## 5. Acknowledgments

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